REMARKS

Claims 12, 14, 17, 19, 29, and 36-41 are pending. Applicants have cancelled claims 1-11, 13, 15, 16, 18, 20-28, and 30-35 and reserve the right to pursue the subject matter in these claims separately. Claims 12, 14, 36, and 38 have been amended herein; the amendments do not introduce any new matter to the pending claims.

Supplemental Information Disclosure Statement

As required by the Examiner, Applicants hereby re-submit the Supplemental Information Disclosure Statement, which includes full references by An et al. and publication dates for Cher and Anderson, as provided by an official email communication from the National Institutes of Health. Additional references are also included in the Supplemental Information Disclosure Statement, which will be discussed in greater detail below. Applicants respectfully request that the Examiner consider these references.

Rejection of Claims 12, 14, 17, 19, 29, and 36-41 Under 35 U.S.C. § 112, First Paragraph

Claims 12, 14, 17, 19, 29, 36-41 stand rejected under 35 U.S.C. § 112, first paragraph for alleged lack of enablement. The Examiner has reiterated her belief that the claims as written are extremely broad, that the specification does not provide adequate guidance and working examples, that the state of the art is highly unpredictable, and that the quantity of experimentation required for a skilled artisan to practice the claimed methods would be enormous.

Applicants maintain that the instant application is directed to an assay for analyzing the expression levels of one or more gene products which Applicants have discovered to be statistically significant markers for predicting the likelihood of the development of a metastatic condition. The present application teaches that by looking at the expression levels of specific gene products, e.g., genes which control the actin-based cytoskeleton, the likelihood of the development of a metastatic condition can be assessed.

Accordingly, the claimed methods are based on the correlation of one or more particular gene products' expression levels with the potential for an individual to develop a metastatic

condition. Applicants respectfully submit that this correlation has been adequately taught by the specification with numerous specific gene product examples and confirmed by more recent studies, as discussed in greater detail below.

The specification further describes how to ascertain differential expression levels of gene products in different samples. Additionally, a skilled artisan would appreciate that other methods well known in the art can be applied, such as immunostaining, RT-PCR, northern blotting, etc., to practice the claimed methods. A skilled artisan would also appreciate the advantages or disadvantages of each method and choose accordingly.

More importantly, the instant application also teaches skilled artisans the specific gene products and how to validate specific gene products, the expression levels of which can be used to predict the likelihood of developing a metastatic condition. Indeed, Applicants have shown several examples of gene products capable of altering actin-based cytoskeleton (e.g., RhoC, thymosin β_4, α-actinin 1, and Hsp70) and their increased expression levels observed in metastases versus primary tumors. Further, Applicants have provided ample guidance on how to validate the involvement of a specific gene product, RhoC, in metastasis and invasiveness of cancer cells. *See, e.g.,* pages 33-34 of the specification, the section entitled "RhoC Enhances Invasive Phenotype." As such, a skilled artisan would appreciate that, with any gene product that may be implicated in tumor metastasis, he/she can following the specification's teachings to determine the expression levels of the gene product in non-metastatic versus metastatic samples and to further determine whether the gene product is essential for metastasis following the guidance as provided in the working examples regarding RhoC in the present application.

Additionally, the metastases described in the working examples of the instant application are lesions obtained from a murine metastasis model, and murine models for studying metastasis are well accepted by skilled artisans. *See, e.g.*, An et al., Anticancer Res. 16:627-31 (1996) and Clin. Exp. Metastasis 15:184-95 (1997); Cher, 1R01CA088028-01 (same as 5R01CA088028-03); Anderson, 5R01CA090291-02, Grant Abstracts, National Cancer Institute, National Institutes of Health. Indeed, the approach described in the present application and Clark et al. has been commented upon by others as follows: "The process of selecting highly metastatic cell lines, as done by Clark et al (2000), resulted in a large number of genes related solely to metastasis . . . ." Margalit et al., British J. Cancer 89:314-19 (2003). The instant application also recognizes this, e.g., at page 31, lines 5-9 of the specification, which compares pulmonary

metastases and subcutaneous tumors developed from the same metastatic tumor cells and finds that the gene expression patterns are intrinsic to the metastatic cells and not affected by the microenvironment of the tumors.

The Examiner is concerned that the specification teaches only results obtained with tumor cell lines. Applicants draw the Examiner's attention to the working examples, in which Applicants describe that the RNAs for measuring gene expression levels with microarrays are obtained from metastases and subcutaneous tumors, that first-round pulmonary metastases (e.g., M1) are obtained by injecting well-recognized tumor cell lines, and that further round of metastases (e.g., M2) are obtained by culturing cells from M1 (which demonstrate higher potential to develop metastasis than the starting tumor cell line). As discussed above, the M2 metastases developed from M1 and subcutaneous tumors developed from M1 show nearly identical gene expression patterns, which indicate that the higher gene expression levels observed in metastases do not depend on the formation of the metastases but rather are intrinsic to the M1 cells that demonstrate a high potential to metastasize.

Moreover, the experimental findings described in the instant application have been published by the highly acclaimed, peer-reviewed scientific journal Nature. Clark et al., Nature, 406: 532-35 (August 2000). The journal Nature even dedicated a concurrent "News and Views" essay to comment on the important findings. Ridley, Nature 406:466-467 (August 2000). The commentator first characterized that "gene-expression profiling, using high-density DNA microarrays, is revolutionizing our approach to studying cancer." The commentator also focused on the fact that Clark et al. chose RhoC among the genes that showed differential expression patterns between human and mouse metastases and their non-metastatic counterparts and "find that, remarkably, overexpression of one of these genes—RhoC—can stimulate metastasis all by itself." The commentator concluded by stating that "the approach taken by Clark et al. . . . does provide an unbiased method by which to pinpoint important, and potentially new, contributors to cancer."

Further, the experimental findings that support the instant application have also been confirmed by many studies published since the filing of the present application. For example, as reviewed by Goldstein, J. National Cancer Institute 95(22):1646-1647 (November 19, 2003), "[u]ntil recently, little attention has been paid to the nuclear activities of actin, a housekeeping protein, or the actin-sequestering molecule thymosin β_4, or to their use as potential targets for

antitumor strategies. . . . It now appears that actin and some of the molecules that regulate actin are *bona fide* residents of the nucleus and may play previously unrecognized roles in tumor metastasis and angiogenesis." Goldstein focused on Cha et al., a concurrent (2003) publication that demonstrated a key role by thymosin β_4 in facilitating tumor metastasis and angiogenesis. Goldstein cited three additional studies that demonstrated elevated expression of thymosin β_4 in metastasis, which included two 2003 publications in Oncogene and Clark et al., the 2000 Nature publication that contained the findings in support of the instant application. Additionally, two recent publications have also confirmed that fibronectin expression correlates significantly with tumor stage in ovarian cancer and lymph node involvement in breast cancer. *See*, Franke et al., Anticancer Research 23:4261-4268 (2003); Ioachim et al., European J. Cancer 38:2362-2370 (2002). Further, recent studies have also demonstrated that the levels of angiopoietin 1 and surfactant protein C, are also elevated in different metastases. *See, e.g.*, Quentin et al., Anticancer Research 24(5A):2745-56 (Sep.-Oct. 2004) (showing that angiopoietin mRNA was expressed at a 3-fold significantly lower level in low-grade, low-stage compared to high-grade, high-stage transitional cell carcinomas (TCC) of the urinary bladder); Margalit et al., British J. Cancer 89(2):314-319 (July 21, 2003) ("Microarray analysis defined seven genes, [including] surfactant protein C (SP-C) . . . , which were consistently elevated in pulmonary metastases compared to the primary tumour of both D122 and B16-F10.9 models.").

Therefore, Clark et al. and the instant application represent significant progresses made in the art, which have been validated by more recent studies. As also discussed above, the specification provides ample guidance on how to practice the claimed methods. Accordingly, Applicants maintain that the specification and the pending claims enable one of skill in the art to practice the application as currently claimed without undue experimentation. Reconsideration and withdrawal of the rejection are respectfully requested.

Rejection of Claims 12, 14, 19, and 36-39 Under 35 U.S.C. §102(a)

Claims 12, 14, 19, and 36-39 stand rejected under 35 U.S.C. §102(a) as allegedly anticipated by Kaur et al. (Oral Oncology 34 (1998) 496-501).

Applicants have amended claims 12, 14, 36, and 38 to exclude Hsp70. Similar to excluding RhoC, excluding Hsp70 from claims 12 and 36 does not introduce any new matter to

the amended claims because Hsp70 was positively recited in the specification and claims as filed.

Kaur et al. teaches that overexpression of Hsp70 is correlated with increased odds for developing nodal metastasis for oral cancer. However, Kaur et al. does not teach or suggest the involvement in nodal metastasis for oral cancer of any other gene product. Accordingly, the amended claims are patentable over Kaur et al., and Applicants respectfully request that the Examiner reconsider and withdraw this rejection.

Rejection of Claims 36-40 and 42 [sic] Under 35 U.S.C. §103(a)

Claims 36-40 and 42 [sic] stand rejected under 35 U.S.C. §103(a) as allegedly unpatentable over Christensen et al. (Cancer Research 1988). Applicants assume that the Examiner intended to reject claims 36-41.

According to the Examiner, Christensen et al. specifically teach that fibronectin levels are an excellent prognostic factor for metastatic potential, and thus that it would have been prima facie obvious to have applied the methodologies taught by Christensen et al. to the prediction of an increased likelihood of developing metastasis.

However, Christensen et al. must be viewed in light of the state of the prior art. As noted by the Examiner in the outstanding Office Action as well as the previous Office Action dated November 7, 2003, the state of the art before the filing date of the present application is such that the expression level of fibronectin gene was not found to consistently correlate with the potential to develop a metastatic condition. For example, at page 10 of the outstanding Office Action, the Examiner cited various prior art references (e.g., Christensen et al. and Takei et al.). Although Christensen teaches that stromal fibronectin (SFN)-positive immuno-staining reaction was significantly associated with a low metastatic potential in samples from invasive breast carcinomas from 77 women, Takei et al. teach that SFN expression did not correlate with lymph node metastases or tumor size based on immunohistochemistry study of 83 breast cancer specimens.

Applicants also draw the Examiner's attention to Christensen, APMIS Supplementum No. 26, Vol. 100: 1-36 (1992), included in the Supplemental Information Disclosure Statement, which states at page 14: "Demonstration of FN immunoreactivity within epithelial breast cells *in*

vivo has been a more inconsistent finding depending on tissue preparation and fixation. . . . Benign epithelia have occasionally revealed an inconsistent staining luminally or preicellularly . . . . and intracellularly . . . , but the most intense reaction has been observed in individually growing tumor cells and in cells from poorly differentiated carcinomas . . . as well as in metastatic cells from lymph nodes . . . " (internal citations omitted). Christensen went on to state, at page 23, that the "significance of FN . . . for the prognosis of breast cancer has not been definitively established."

Thus, Christensen et al., taken together with knowledge in the prior art, teaches away from relying on the level of fibronection expression to predict the likelihood of developing a metastatic condition. With the results obtained by highly sensitive microarray technology and stringent selection criteria as disclosed in the present application, however, a skilled artisan can now rely on the level of fibronection expression to carry out the claimed methods. As discussed above, the results disclosed in the present application have been confirmed with more recent studies published after the filing date of the present application. *See* Franke et al. (Anticancer Research 23:4261-4268 (2003)) and Ioachim et al. (European J. Cancer 38:2362-2370 (2002)).

Further, the pending claims 14 and 29 are directed to methods based on the experimental findings described in the instant application that increased expression of the fibronectin gene correlates with increased potential for an individual to develop metastasis. In contrast, Christensen et al., as well as other earlier studies such as Xu et al., and Guo et al., teaches the opposite, that is, they reported that increased expression of the fibronectin gene correlates with decreased metastasis potential. Therefore, the cited reference, Christensen et al., teaches away from the claimed methods.

Accordingly, Applicants respectfully submit that the pending claims are patentable over Christensen et al. and request that the Examiner reconsider and withdraw the rejection.

CONCLUSION

In view of the above, each of the presently pending claims in this application is believed to be in condition for immediate allowance. Accordingly, the Examiner is respectfully requested to pass this application to issue. If the Examiner feels that a telephone conference would

expedite prosecution of this case, the Examiner is invited to call the undersigned at (617) 951-7000.

If any additional fee is due, please charge our **Deposit Account No. 18-1945**, from which the undersigned is authorized to draw under **Order No. WIBL-P01-534**.

Dated: March 22, 2005

Respectfully submitted,

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